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ASPECTS OF THE COURTSHIP BEHAVIOR OF THE BLACK WIDOW SPIDER, *LATRODECTUS HESPERUS* (ARANEAE: THERIDIIDAE), WITH EVIDENCE FOR THE EXISTENCE OF A CONTACT SEX PHEROMONE

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ABSTRACT

The courtship and mating behavior of the black widow spider, *Latrodectus hesperus* Chamberlin and Ivie, were studied to determine stimuli responsible for mate location and courtship initiation in this species. We observed new courtship patterns which included a vigorous display performed by females, "push-ups" executed by both sexes, and cryptic abdominal vibrations produced by males immediately upon contact with female webs. Males initiated courtship when they contacted unoccupied conspecific female webs, but did not respond when placed on other male webs. Male *L. hesperus* also initiated courtship behavior on unoccupied female webs of another species, *Latrodectus mactans* (Fabricius). Female *L. hesperus* were stimulated by contact with conspecific male webs, but not other female webs. Scanning electron microscopy revealed what are presumed to be chemoreceptive hairs on the tarsi and pedipalps of males and females. We conclude that male and female *L. hesperus* produce sexually specific, complementary contact pheromones which are incorporated into their silk. These substances apparently function in mate location, sex identification, and courtship for this species, but not as an isolating mechanism between *L. hesperus* and *L. mactans*.

INTRODUCTION

Courtship and mating behavioral patterns of black widow spiders, *Latrodectus* spp., have been described by Herms et al. (1935) and D'Amour et al. (1936). Kaston (1970) added important details to these descriptions; however, questions concerning mate location and the stimuli responsible for causing males to initiate courtship remain unanswered.

Montgomery (1910) and Hewitt (1917) first suggested that contact-chemical and tactile cues may be important in sexual and species recognition by nocturnal web-building arachnids. Subsequently, Gerhardt (1924), Locket (1926) and Bristowe (1929) observed that male web-spinners were sexually stimulated upon contact with the female's web. Experiments on vagabond spiders led Kaston (1936) to conclude that both contact chemoreception and visual clues were used in mate location by members of this group. Hegdekar and Dondale (1969) demonstrated the existence of a contact sex pheromone in the threads of lycosid spiders. Their study revealed that the pheromone is species-specific,

stable in air, and secreted only by adult females, regardless of their prior mating experience. Blanke (1973) examined the sexual behavior of the tropical Araneid *Cyrtophora cicatrosa* and found that males were attracted to empty bags previously occupied by females, indicating an olfactory sexual pheromone. No data are available on the cues used by males of any species of *Latrodectus* to locate females, or on the stimuli responsible for releasing courtship behavior.

Several workers have searched for chemoreceptors in spiders. Kaston (1935) presented evidence suggesting that the slit or lyriform organs were chemosensory, but electrophysiological experiments later showed these structures to have a mechanoreceptive function (Walcott and Van der Kloot 1959). Blumenthal (1935) indicated his belief that the "tarsal organs" were chemoreceptors; however, these structures occur on the proximal ends of tarsi and palps and would, therefore, not normally contact chemically active substrates. Foelix (1970) identified curved, blunt-tipped hairs on the legs of spiders which he presumed to be chemoreceptors. These hairs featured an open tip to a lumen and were distributed on all tarsal segments. That they closely resemble chemosensitive hairs on the antennae of insects and were found in all spiders studied, seems quite persuasive. No species of black widow spider was among those Foelix examined.

This study was initiated to determine the role of contact chemoreception in the reproductive behavior of *Latrodectus hesperus* Chamberlin and Ivie. We report previously underscribed male and female courtship patterns which were used in experiments to behaviorally bioassay the chemical activity of webs. We also present scanning electron micrographs of *L. hesperus* tarsi which reveal what are presumed to be contact chemoreceptors.

GENERAL METHODS

Latrodectus hesperus of both sexes and all developmental stages were collected in the fall of 1977 in Phoenix and Tucson, Arizona. Four specimens of *Latrodectus mactans* (Fabricius) were acquired from east central Oklahoma, near Okemah. Individuals were isolated in 30 x 70 mm amber plastic vials in the laboratory and fed mealworms (*Tenebrio* sp.) and pink bollworm larvae (*Pectinophora gossypiella*). Courtship was staged in 11 x 12 cm plastic containers and the chemical activity was behaviorally bioassayed on those webs constructed in plastic vials. More than 25 courtship encounters were staged and observed. Unless otherwise indicated, all experiments were conducted on a sample size of seven. Individuals used in experiments were tested once in 24 hours. Male and female tarsi and pedipalps were mounted on aluminum pegs with double sticky cellophane tape, vacuum coated with gold-palladium, and observed on an Etec Autoscan scanning electron microscope. Scanning electron micrographs were produced with Type 55 pos-neg Polaroid film.

RESULTS

Courtship.—The courtship and mating behavior of *L. hesperus* generally conformed to the descriptions of previous workers. A synthesis of previous accounts and our observations follows: Males charged their palpal organs with semen shortly after the definitive molt. Thus equipped for mating, they generally showed heightened levels of activity over earlier instars. They abandoned their webs and no longer actively captured prey. (One male was observed to feed on the captured prey of a female in her web). Upon entering a

female's web, the typical male began his courtship display which consisted of tapping and tweaking the lines with his front tarsi. While so doing, the male explored the web cautiously, rhythmically tapping the silk with his pedipalps. During this period of exploration, the male's body jerked spasmodically and the abdomen was vibrated at a high frequency. Periods of rest interspersed active searching and display. The female frequently initially rejected the display and charged the male, in which case the male beat a hasty retreat or silked from the female's web.

Approaching the female cautiously, the male usually cut the web at strategic points, effectively reducing the female's potential routes of escape. At this point, the courting male caressed the female's legs, then her abdomen, and ultimately climbed excitedly over her body (Figs. 1a and b). A courting male was frequently observed to "throw silk" about the female, forming the so-called "bridal veil." He then positioned himself venter to venter with his mate. Successful males then located the female epigynum and inserted first one, then the other, palpal organ. The time consumed in courtship was highly variable, with a range of from 10 minutes to 2 hours. Males that succeeded in insemination lingered in the vicinity of their mates or wandered leisurely away. This was in marked contrast with the initial cautious approach and escape strategies characteristic of males prior to insemination. Only one male of those we observed to succeed in inseminating a female was eaten by his mate immediately after mating. However, several were later found dead in their mates' webs.

Gerhardt (1924) and others (see Kaston 1970) have indicated that the male loses the distal end of the embolus during copulation. This loss may subsequently render the male impotent. If this is the case, successful males would best serve their biological interests by presenting themselves to their mates as a post-nuptial meal. In so doing, a male would contribute to the production of eggs that he would posthumously fertilize. If the female is sufficiently well-fed to decline this offer immediately after copulation, the male should have no better purpose than to linger on the web until such time as the female's appetite returned.

New Courtship Patterns.—We observed several previously undescribed behavioral patterns in the courtship of *L. hesperus*. Not previously noted was occasional extreme aggressivity of females in courtship. Eager females exhibited jerky movements and repeated violent twitches of the abdomen. These patterns were identical to male courtship behavior but they appeared to be more violent, owing to the much larger size of the female. Males responded positively to female displays and these events usually resulted in successful insemination of the female.

Both sexes were consistently observed to execute "push-ups" (i.e., alternate flexion and extension of the legs) while courting, but this pattern also routinely occurred in the context of disturbance. While viewing males through a dissection microscope, we noted that the male abdomen usually began vibrating shortly after the individual had made contact with the female's web. These vibrations had escaped our preliminary unaided observation. We also noted that shortly after the onset of abdominal vibration, and before they had contacted the female, males began to "throw silk" with their hind legs. Furthermore, males trailed silk while searching the female web. Subsequent experiments revealed that abdominal vibration and silk throwing occurred on webs unoccupied by female spiders, suggesting that males were responding to contact with the web in the absence of visual clues. We, therefore, suspected the presence of a contact pheromone in the web of the female and designed experiments to test this hypothesis.

Contact Pheromone Experiments.—Abdominal vibrations and body tremors of adult male and female *L. hesperus* were used in behavioral bioassay experiments to determine the presence of a contact pheromone in the web of this species. The occurrence of these patterns was scored as a positive response. If these patterns did not occur, a negative response was noted.

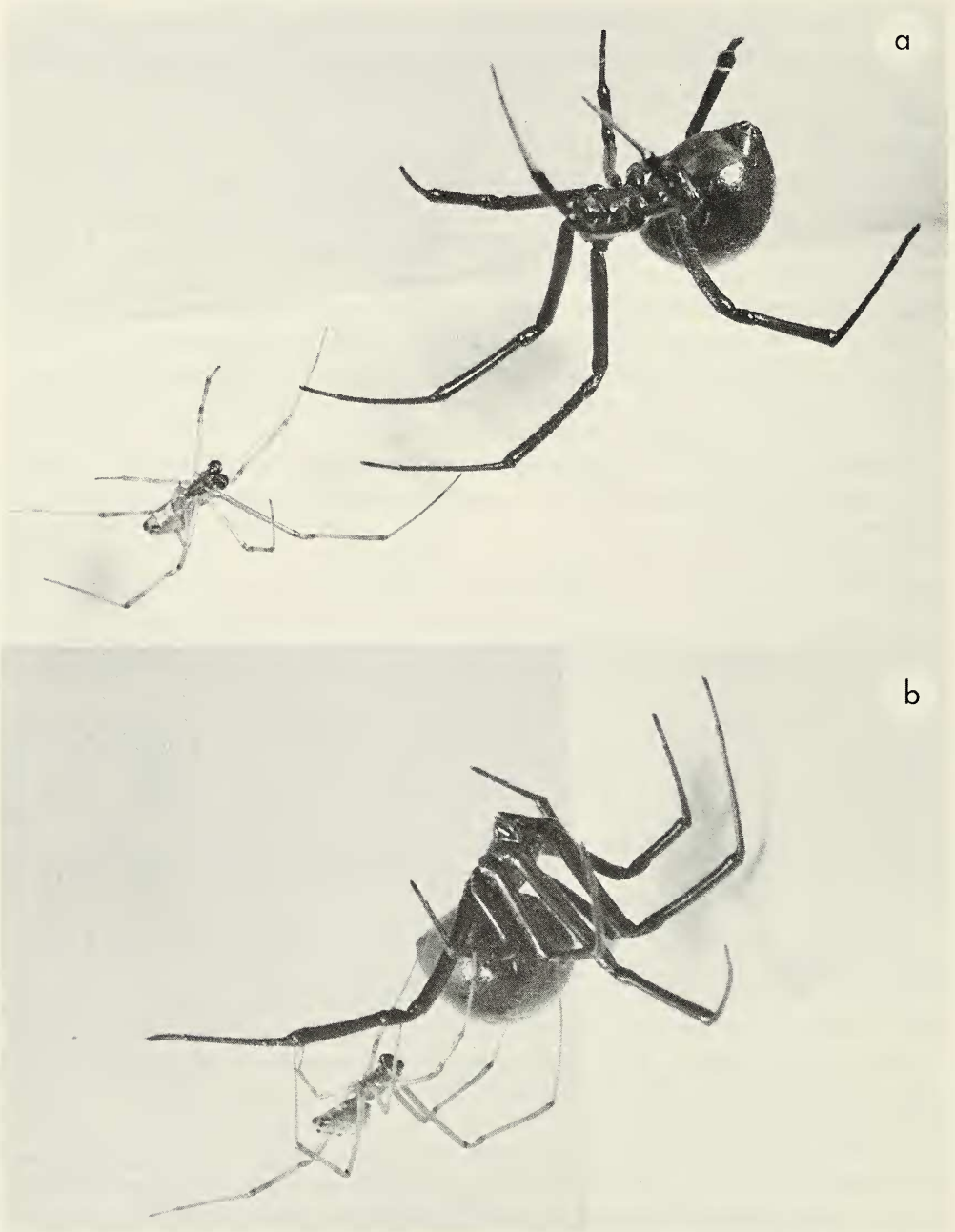


Fig. 1.—Contact between a courting pair of black widow spiders: 1a, leg to leg contact (direct tactile stimuli are exchanged in this essential step); 1b, leg to body contact (during this step the male may wrap the female with his threads).

Male spiders ($n=7$) were placed on male webs from which the original occupants had been removed. Each was observed for 10 minutes. In no case did the tested males show indications of sexual excitement, but the introduced males actively explored the webs on which they had been placed. Adult males ($n=7$) were placed on unoccupied female webs and observed as before. All males responded positively (i.e. with abdominal vibrations) shortly after contact with the female web.

We next attempted to determine if males were responding to the physical structure or chemical content of the female webs. Adult female webbing was removed from containers and compressed into small balls to disrupt its physical properties. These were then placed into vials into which candidate males were introduced. All males tested exhibited signs of sexual excitement when they contacted compressed balls of a female web. This result seemed to support the contact pheromone hypothesis.

We attempted to determine how long the female webs would retain their ability to stimulate males. Unattended webs were left exposed to open air and males were tested daily on these. The results of this experiment were variable (Table 1). Three males continued to be sexually stimulated on webs that had been exposed to air for over 2 weeks. In one case, a male responded to a vacant female web for 50 days, at which time he died. Four of the unoccupied female webs elicited a male response for no longer than three days.

We next tested the activity of webs produced by immature females. Males were placed on unoccupied webs of fourth and fifth instar females and observed as before. In all but one trial, the males reacted positively, although their behavior patterns seemed to be qualitatively less vigorous than those exhibited on adult female webs.

An alternative to the contact pheromone hypothesis was that males were responding to an airborne chemical emanating from the female or her web. To test this possibility, a female was placed in a clean vial for 5 minutes, at which time she and any webbing she had produced were removed. A male was then introduced immediately into the vial. In no case did the male ($n=7$) respond positively. A refinement of this experiment involved placing a partition of perforated aluminum foil over the female and her web in the vial. Males were then placed on top of the perforated foil and observed. None of the males ($n=7$) tested exhibited sexual excitation.

Sexual pheromones have been shown to function in species isolation for a large number of insect species (Jacobson and Beroza 1963). We wondered if the chemical present in the web of *L. hesperus* was species-specific. To check this possibility, we placed an *L. mactans* adult male on seven different webs of female *L. hesperus*. Before each trial, an *L. hesperus* male was used to validate the activity of the conspecific webs. All webs elicited a positive response from *L. hesperus* and surprisingly also from the *L. mactans* male. A modified reciprocal of this experiment was conducted. Male *L. hesperus* ($n=7$) were placed on the webs of *L. mactans*. No *L. mactans* males were available to test the activity of conspecific female webs, so *L. hesperus* were tested first on the nonspecific web, then for the sake of comparison, on the webs of *L. hesperus* females. *L. hesperus* males reacted positively to all webs, but showed a qualitatively more vigorous response to conspecific webs.

Finally, we wished to determine the response of females to conspecific male webs. All females ($n=7$) exhibited the excited movements previously observed in response to male courtship. This behavior was again strikingly similar to the courtship behavior of the males. As a control, females were placed on the unoccupied webs of other females. In three trials, the females showed no reaction whatsoever. Four of the seven responded by

Table 1.—Duration of sexual responsiveness of males to webs of females exposed in open air.

Specimens tested	Duration of sexual response
Male 212 on web of female 17	3 days
Male 210 on web of female 1	2 days
Male 206 on web of female 19	14 days
Male 206 on web of female 10	2 days
Male 206 on web of female 7	2 days
Male 224 on web of female 406	50 days (male died)
Male 202 on web of female 413	16 days

exploring the new web for a short period of time. The exploratory behavior lacked the components which were elicited in response to contact with male webs.

Chemoreceptors.—Scanning electron micrographs of the tarsi and pedipalps of *L. hesperus* revealed what are presumed to be the chemosensory hairs described by Foelix (1970) for other web-building spiders (Figs. 2b and c). These are arranged in parallel rows pointed toward the distal end of the appendages and inserted at an angle of about 80° to the axis of the leg. They are blunt (open) tipped, slightly curved structures, etched in a spiral pattern. The hairs measure 50-60 μm in length, and have a diameter of ca. 5 μm at the base and 2 μm at the tips. Each is inserted in a crater-like socket. These structures are uniformly distributed on all tarsal segments, (Fig. 2a) and on the ventral surface of the palpal organs in both sexes. These are the anatomical parts which contact the web during courtship.

The presumed chemoreceptors are distinct from mechanoreceptors, in that the latter are at least five times longer than the former. Also, the mechanoreceptive hairs form an angle of less than 30° with the axis of the appendage, and they are inserted on conical processes. They are sharp-pointed rather than blunt, and have no end opening.

DISCUSSION

Our data strongly suggest that *L. hesperus* males locate conspecific females and identify potential mates by contact with the female web. Furthermore, it is apparently a chemical component or components of the web and not its physical structure to which males are responding. This system surely functions to the advantage of both sexes. The female spider's web effectively extends her appendages several hundred times in length, and the volume of space she commands by an equivalent factor. If in the evolution of these spiders, some females produced chemicals (perhaps metabolic wastes) and excreted these into their silk, and some males in the population possessed receptors tuned to these chemical cues, both the producers and recipients of this information would have enhanced probability of meeting and mating. This mutual advantage has apparently resulted in selection favoring co-evolutionary refinement of the system.

Vagabond spiders of the families Salticidae, Pisauridae, and Thomisidae, phylogenetically subordinate to the Theridiidae, do not construct webs and rely on direct contact to exchange chemical stimuli important in species and sexual recognition (Kaston 1936). Wolf spiders (Lycosidae), a somewhat more highly evolved family, illustrate a

possible transitional condition. They rely on direct contact, and females produce small temporary threads which contain contact sexual pheromones (Kaston 1936, Hegdekar and Dondale 1969). The system of web-borne contact chemical communication may reach its highest development in the Araneidae, the orb weavers, a group characterized by the production of very large and elaborate webs.

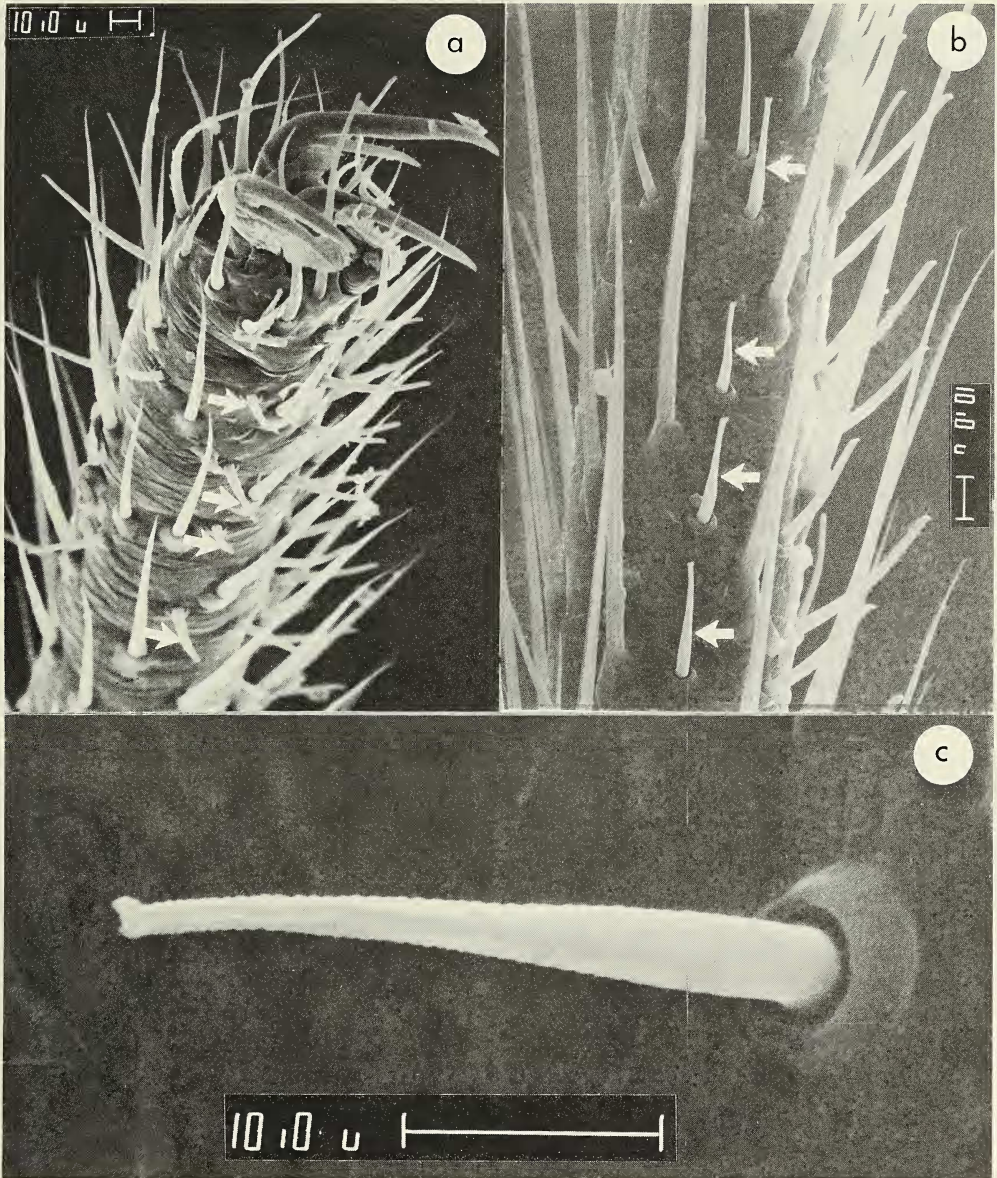


Fig. 2.—Scanning electron micrographs of structures on adult male black widow spiders: 2a, apical tarsal segment showing row of presumptive contact chemosensitive hairs (indicated by arrows) towards the apex of the tarsus; 2b, curved, blunt-tipped presumptive chemosensitive hairs (arrows) occur in longitudinal rows, with rows of pointed mechanoreceptive hairs between them; 2c; close-up of an individual presumptive chemosensitive hair, note spiral texture, open tip, and crater-like insertion.

We were somewhat perplexed that the contact pheromone in the web of *L. hesperus* is apparently not distinct from that produced by the closely related species, *L. mactans*. The precise distribution of the two species is not known, however, Kaston's (1970) distributional notes suggest that the two species are allopatric; hence, there has been no selection favoring divergence in the chemical components between the two. Also, it is unlikely that the pheromone produced by the two species would chemically differentiate by genetic drift if they consist of essentially unmodified metabolites. Significantly, Kaston (1970) was able to stage three successful matings between *L. mactans* and *L. hesperus* in the laboratory, indicating that premating isolating mechanisms between the two are not highly developed. We assume that the active substances in female webs are sufficiently different from those of other less closely related species in the family and members of other families so that male black widows are able to avoid dangerous and wasteful courting on heterospecific webs.

Another problem arises from our discovery that the webs of sexually immature and mated adult females as well as those of adult virgins are chemically active. This condition might work to the detriment of males because it would cause them to waste time courting unreceptive females. Chemically active webs could, on the other hand, be of considerable benefit to their immature or previously mated adult female occupants in that courting males are potential prey. The initial cautious behavior of males on conspecific female webs would seem to reveal their innate awareness of this danger. Indeed, Bristowe (1929) speculates that the marked sexual dimorphism manifest in web spinners is an adaptation that enhances male agility and escape potential on female webs.

Kaston (personal communication) has suggested another explanation for the apparent chemical activity of immature female webs. A male that contacts an immature female web may (cautiously) linger on it until the female has undergone the definitive molt, at which time the patient male would be rewarded with a receptive potential mate. This system would be mutually advantageous to both sexes. It might be particularly advantageous to the male if there is high risk involved in searching for female webs. This hypothesis is clearly in need of further study. (Subsequent to Dr. Kaston's communication, we have observed four instances of adult male *L. hesperus* waiting on immature conspecific female webs in the field. Although we did not see mating, in each case males remained unmolested on immature female webs for several days).

Abdominal vibrations by both sexes seem to be important initial components in courtship. The male apparently immediately announces his presence on the female web using this behavioral pattern and his mechanical message is communicated via the web to the female. Females almost certainly use this information to distinguish courting males from captured prey. The female, if she is receptive, responds to the male vibrations in kind, thus encouraging the male.

Female responsiveness to male webs revealed by our experiments seemed anomalous in that females probably do not search for males, and adult males do not construct webs. Reproductive males do, however, produce silk while courting. It may be that in close encounters between the sexes, females chemically validate the identity of the male by contacting the silk he throws to produce the "bridal veil." It was apparent to us that the "bridal veil" symbolically, rather than physically, binds the female. The female could easily break the binding threads, but may be inhibited from doing so by her perception of their chemical content, a possible complementary male pheromone.

To summarize, it appears likely that black widow spiders incorporate complementary contact sexual pheromones into their silk and that these are detected by chemoreceptors

in the legs and palps of the opposite sex. The female-produced pheromone serves to aid the male in gross location of a conspecific mate and releases his initial courtship behavioral patterns. The male-produced pheromone inhibits the female's predatory response and probably lowers her threshold for mating readiness.

ACKNOWLEDGMENTS

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